African Journal of Biotechnology Vol. 11(87), pp. 15291-15302, 30 October, 2012

Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1036
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Genetic diversity of Kenyan *Prosopis* populations based on random amplified polymorphic DNA markers

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Accepted 10 August, 2012

Several *Prosopis* species and provenances were introduced in Kenya, either as a single event or repeatedly. To date, naturally established *Prosopis* populations are described as pure species depending on site, despite the aforementioned introduction of several species within some sites. To determine whether naturally established stands consist of a single or mixture of species, six populations from Bamburi, Bura, Isiolo, Marigat, Taveta and Turkwel were compared for relatedness with reference to *Prosopis chilensis*, *Prosopis juliflora* and *Prosopis pallida* using random amplified polymorphic DNA markers. Cluster analysis based on Nei's genetic distance clustered Kenyan populations as follows: Marigat, Bura and Isiolo with *P. juliflora,* Bamburi with *P. pallida* and Taveta with *P. chilensis,* whereas the Turkwel population is likely to be a hybrid between *P. chileneis* and *P. juliflora*. Four populations had private markers, revealing germplasm uniqueness. Expected heterozygosity tended to be larger for Kenyan populations (ranging from 0.091 to 0.191) than in the three reference (ranging from 0.065 to 0.144). For the six Kenyan populations and two *P. juliflora* provenances from the Middle East, molecular variation was larger within populations than between population. Higher molecular variance among populations is attributed to their geographical separation and the low variation within populations is due to gene flow between individuals within a population. Overall, this study shows that (1) the Kenyan *Prosopis* populations are genetically isolated, (2) multiple introductions enhanced genetic diversity within sites and (3) *P. juliflora* and its hybrid are the most aggressive invaders.

Key words: *Prosopis chilensis, Prosopis juliflora, Prosopis pallida*, multiple introductions, genetic diversity.

INTRODUCTION

The genus *Prosopis* Linnaeus emend. Burkart has 44 species of trees and shrubs found in the hot dry tropics of America, Africa and Asia (Burkart, 1976). About 90% of all *Prosopis* species are native to North and South America. Taxonomically, the genus is classified into Algorobia (30 species), Anonychium (1 species), Monilicarpa (1 species), Strombocarpa (9 species) and Prosopis (3 species) sections (Burkart, 1976). Some species within a section (Burkart, 1976), as well as their hybrids, are morphologically indistinguishable (Saidman et al., 1996; Vega and Hernandez, 2005). Consequently, species misidentification is common, particularly in areas of *Prosopis* species introductions (Harris et al., 2003; Landeras et al., 2006). Proper identification of species is required for species-specific invasion management, for example through biological control (Zimmerman, 1991; van Klinken, 1999).

In Kenya, eight *Prosopis* species were periodically introduced to various sites (Maghembe et al., 1983; Rosenschein et al., 1999; Stave et al., 2003; unpublished data from the Kenya Forestry Seed Centre). Introduced species are from the sections Algarobia (*P. alba* Griseb., *P. chilensis* Stuntz, *P. juliflora* (Sw.) DC*, P. nigra* Hieron,

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and *P. pallida* Kunth), Strombocarpa (*P. pubescens* Benth. and *P. tamarugo* Phil.) and Prosopis (*P. cineraria* Druce). *Prosopis* species and provenances have been introduced sometimes in a single event, but often by multiple introductions (Kaarakka et al., 1990; Otsamo et al., 1993; Oba et al., 2001). The introduced species have adapted and become invasive, causing negative socio economic and ecological impacts (Stave et al., 2003; Mwangi and Swallow, 2008; Mworia et al., 2011).

Multiple introductions can enhance species invasiveness in four ways: through propagule pressure, colonization pressure (Lockwood et al., 2009; Blackburn et al., 2011), hybridization, and genetic diversity (Shierenbeck and Ellstrand, 2009; Pairon et al., 2010). Propagule pressure is an inclusive term for frequency, size, spatial and temporal patterns of propagules arrival when dealing with a single species. On the other hand, colonization pressure is commonly used in reference to the frequency, size, spatial and temporal patterns of propagules arrival in reference to several species. For a species, the probability to establish a viable population increases with propagule pressure, whereas that of species increases with colonization pressure (Lockwood et al., 2009; Simberloff, 2009). When the barrier between geographically separated species or provenances is removed through introduction of species and provenances, hybridization and subsequent gene introgression may occur, thus increasing genetic diversity and enhancing the adaptability of progenies (Parsons et al., 2011). For example, hybrids have compared to their progenitors, an expanded habitat range in the case of sunflower (Rieseberg et al., 2007), higher growth rate in the case of *Mahonia* species (Ross and Auge, 2008), and higher seed germination and seedling growth rates in the case of *Schinus terebinthifolius* Raddi (Geiger et al., 2011). In combination, these traits may enhance the invasiveness of plant hybrids.

The introduction of *Prosopis* species in Kenya was characterized by both propagule pressure through multiple introduction of *P. chilensis, P. juliflora* and *P. pallida* provenances in sites such as Bura (Kaarakka et al., 1990; Otsamo et al., 1993), and colonization pressure by multiple introduction of several species within specific sites (Maghembe et al., 1983; Oba et al., 2001; Rosenschein et al., 1999; unpublished data from the Kenya Forestry Seed Centre). The pooling of germplasm at introduction, their subsequent exchange between sites (Kaarakka et al., 1990; Otsamo et al., 1993) and random seed dispersal by livestock, wildlife and water (Mwangi and Swallow 2008; Mworia et al., 2011) could have enhanced hybridization and gene introgression, which are known to occur among the *Prosopis* species (Bassega et al., 2000; Vega and Hernandez 2005; Landeras et al., 2006). To date, the composition and diversity of introduced *Prosopis* species in Kenya is largely unknown. There is a general tendency to classify populations of invaded areas as *P. juliflora* (Pasiecznik et al., 2001; Ngunjiri and Choge, 2004; GoK, 2007; Trenchard et al., 2008). Populations have also been classified as either *P. chilensis* (Stave et al., 2003; Olukoye et al., 2003) or *P. juliflora* (Maghembe et al., 1983; Mwangi and Swallow, 2008) depending on site; whereas other introduced species are occasionally mentioned (Maghembe et al., 1983; Rosenschein et al., 1999).

Molecular techniques have been successfully used for *Prosopis* species to resolve species identity and progenitors (Vega and Hernandez, 2005; Landeras et al., 2006; Sherry et al., 2011). Such techniques are handy when morphological species identification is problematic (Harris et al., 2003; Landeras et al., 2006). Random amplified polymorphic DNA (RAPD) is a most widely used molecular technique. The genetic diversity of the species is described by heterozygosity, polymorphism and molecular variance (Juarez-Munoz et al., 2002; Ferreyra et al., 2010). The objectives of this study were to quantify genetic diversity of various *Prosopis* populations in Kenya, and identify to what species they belong. We hypothesized that: (a) Kenyan *Prosopis* populations are genetically diversified because of multiple introductions; (b) within sites, populations consist of a mixture of introduced species or species and their hybrids

MATERIALS AND METHODS

Description of sample populations, plant sampling and reference species

Six naturally established *Prosopis* populations (Bamburi, Bura, Isiolo, Marigat Taveta and Turkwel) were selected for sampling (Table 1, Figure 1), based on the literature (Ngunjiri and Choge, 2004; Anderson, 2005; Mwangi and Swallow, 2008) and our knowledge on *Prosopis* distribution in Kenya. Bura, Marigat and Turkwel are heavily invaded areas (Ngunjiri and Choge, 2004). Isiolo is encroached by *Prosopis* species but the encroachment is not yet problematic (Mwangi and Swallow, 2008). During sampling, Taveta was found to be under intermediate invasion threat, whereas Bamburi had mixed exotic and indigenous species used to rehabilitate abandoned limestone quarry mines (Maghembe et al., 1983). Thus, this study ranked the invasion status in the sampled populations as heavy (Bura, Marigat and Turkwel), intermediate (Taveta), low (Isiolo) and no invasion (Bamburi).

30 trees were sampled per site and a distance of ≥ 500 m between trees was used to maximize genetic diversity within a population. The distance between trees was determined with a global positioning system. Young tender healthy leaves were collected from each tree and preserved in polythene bags containing silica gel. All samples were stored in a cool box before being transferred to the laboratory where they were preserved in a deep freezer at -40°C until further analysis to isolate DNA. Seeds of known provenances of *P. chilensis* (batch number FAO 01590/86, provenance Agua Chica), *P. juliflora* (batch numbers 0101594 (Oman-Muscat), 0103738 (Yemen-Abyan) and 0109132 (Venezuela, Nueva Esparta - Isla de Margarita) and *P. pallida* (batch number FAO 01353/84, provenance Zana) were also included in the analysis. With the exception of the *P. juliflora* provenances from Oman and Yemen (Middle East), the reference materials originated from the natural range of the three *Prosopis* species (Burkart, 1976). *P. chilensis* and *P. pallida* seeds were

Table 1. The source of study materials in Kenya and that of reference species as stated by seed supplier (site), number of species introduced to sampled site or reference species description (species), representative geographical location of a sample tree within a site in Kenya (Location) and corresponding elevation (altitude). Sample references are included in the species column describing the species introduced to Kenya sites, whereas reference seed batch numbers for reference species were provided by the seed suppliers.

Site	Species	Location	Altitude (m.a.s.l)
Bamburi	*Prosopis juliflora* and *P. pallida,* Maghembe et al. (1983)	4.02° S, 39.72° E	13
Bura	*P. chilensis*, *P. juliflora* and *P. pallida,* Kaarakka et al. (1990)	1.17° S, 39.85° E	101
Isiolo	*P. juliflora*	0.39° N, 37.67° E	1047
Turkwel	*P. chilensis* and *P. juliflora,* Oba et al. (2001)	3.04° N, 35.50° E	526
Marigat	*P. chilensis, P. juliflora* and *P. pallida,* Rosenschein et al. (1999)	0.47° N, 36.07° E	985
Taveta	*P. juliflora,* Ngunjiri and Choge (2004)	3.42° S, 37.72° E	727
Chile	*P. chilensis* - FAO 01590/86	-	-
Peru	*P. juliflora* - 0101594 , KEW	-	-
Oman: Muscat	*P. juliflora* - 0109132, KEW	-	-
Yemen: Abyan	*P. juliflora* - 0103738, KEW	-	-
Venezuela: Nueva Esparta - Isla de Margarita	*P. pallida* - FAO 01353/84	-	-

obtained from University of Copenhagen, Denmark and *P. juliflora* seeds were obtained from Kew Botanical Gardens, UK. Seedlings for the three species were raised at KEFRI greenhouse and their leaves were sampled for DNA analysis. The three known species were used as reference materials. Reference materials are briefly described in Table 1 and their approximate geographical location is shown in Figure 1. Subsequently, all reference provenances and the Kenyan samples are treated as population.

DNA isolation

DNA isolation was carried out using a modified sodium dodecyl sulphate (SDS) method with RnaseA addition (Edwards et al., 1991; Machua et al., 2011). About 0.1 g of leave tissue was obtained from the tree leaves by shutting an eppendolf (1.5 ml) lid on the leaf to obtain equal leaf discs. Some sterile sand, polyvinylypyrollidone (pvp), 200 µl of SDS extraction buffer [1 M Tris (pH 7.5), 5 M NaCl, 0.5 M EDTA, 10% SDS and 7 µl of mercaptol ethanol] were added, and the samples ground in liquid nitrogen using a sterile plastic micro pestle. An extra 500 µl of the SDS extraction buffer was added and samples were vortexed for 10 s and then left at room temperature for about 45 min. The samples were centrifuged at 10000 rpm for 10 min and 500 µl of the supernatant was transferred into a fresh eppendolf tube and an equal volume of chilled chloroform: isoamyl alcohol (24:1) was added. The samples were mixed well by inversion to emulsify and then centrifuged at 10000 rpm for 10 min after which 400 µl of the supernatant was transferred into a fresh eppendolf tube, and an equal volume of chilled isopropanol added. The samples were then mixed well by inversion and then left at room temperature for about 2 min, followed by centrifuging at 10000 rpm for about 8 min to pellet the nucleic acids while the supernatant was poured off.

The DNA pellet was washed with 0.4 ml of chilled 70% ethanol by centrifuging at 10000 rpm for 1 min. Ethanol was then drained by inverting the tubes and the DNA pellet was re-suspended in 200 µl of TE buffer [10 mM Tris (pH 7.5), 1 mM EDTA]. Thereafter, 2 µl of RNAseA (10 mg ml^{-1}) was added into each sample and the samples incubated at 37°C for about 30 min. A further 2 volume (400 µl) of 99% chilled ethanol was added into each sample and then centrifuged at 10000 rpm for 10 min to re-precipitate the DNA pellets dried under vacuum before re-suspending in 1 ml of TE buffer [10 mM Tris (pH 7.5), 1 mM EDTA] and stored at -20°C before use.

Random amplified polymorphic DNA (RAPD) assay

A total of 40 decamer primers were screened for polymerase chain reaction (PCR) on a batch of *Prosopis* DNA samples. Ten primers revealed clear, reproducible bands and these were selected for amplification of all the samples (Table 2). DNA amplification was carried out in a 25 µl volume reaction mix containing 200 mM of each of the dNTPs (Invitrogen), 1 µl *Taq* polymerase buffer (Invitrogen), 3 mM $MgCl_2$ (Invitrogen), 0.2 M primer (Invitrogen), 2.5 ng l^{-1} DNA and 0.75 units of *Taq* polymerase (Invitrogen). Amplification program included 1 cycle at 15 min, at 94°C (denaturation), 1 min at 36°C (annealing) and 2 min at 72°C (extension). A final 5 min extension (72°C) was allowed to ensure full extension of all amplified products. Amplification products were mixed with 6× gel loading dye (0.25% bromothymol blue, 25% xylene cyanol and 30% glycerol) and separated on a 2% agarose gel. To either side of the gel, 5 µl of 100 bp molecular marker ladder (Invitrogen Ltd) was added to size up the amplified loci. Gels were stained in ethidium bromide and visualized under ultra violet light and photographed using Kodak ID 3.5 gel imaging system (Kodak).

Data analysis

Amplified products were scored for presence (1) or absence (0) of a band using Kodak ID 3.5 application program (Pizzonia, 2001). Data were subjected to genetic analysis using POPGENE 3.2 (Yeh et al., 1999) and GenAlEx 6 (Peakall and Smouse, 2006), assuming diploid inheritance and Hardy-Weinberg equilibrium (Wright, 1976). Genetic distances between populations were calculated according to Nei (1978). Cluster analysis based on Nei's genetic distance was carried out using unweighted pair-wise group arithmetic averaging (UPGMA) method (Sneath and Sokal, 1973), using TFPGA Software (Miller, 1997). Cluster analysis was complimented by Principal component analysis (PCA) on all populations to obtain more insight on distances among populations (Sneath and Sokal, 1973; Hauser and Crovello, 1982). Analysis of molecular variance (AMOVA) was carried out to partition genotypic variance among the 3 regions (Kenya, Middle East and South America). The AMOVA

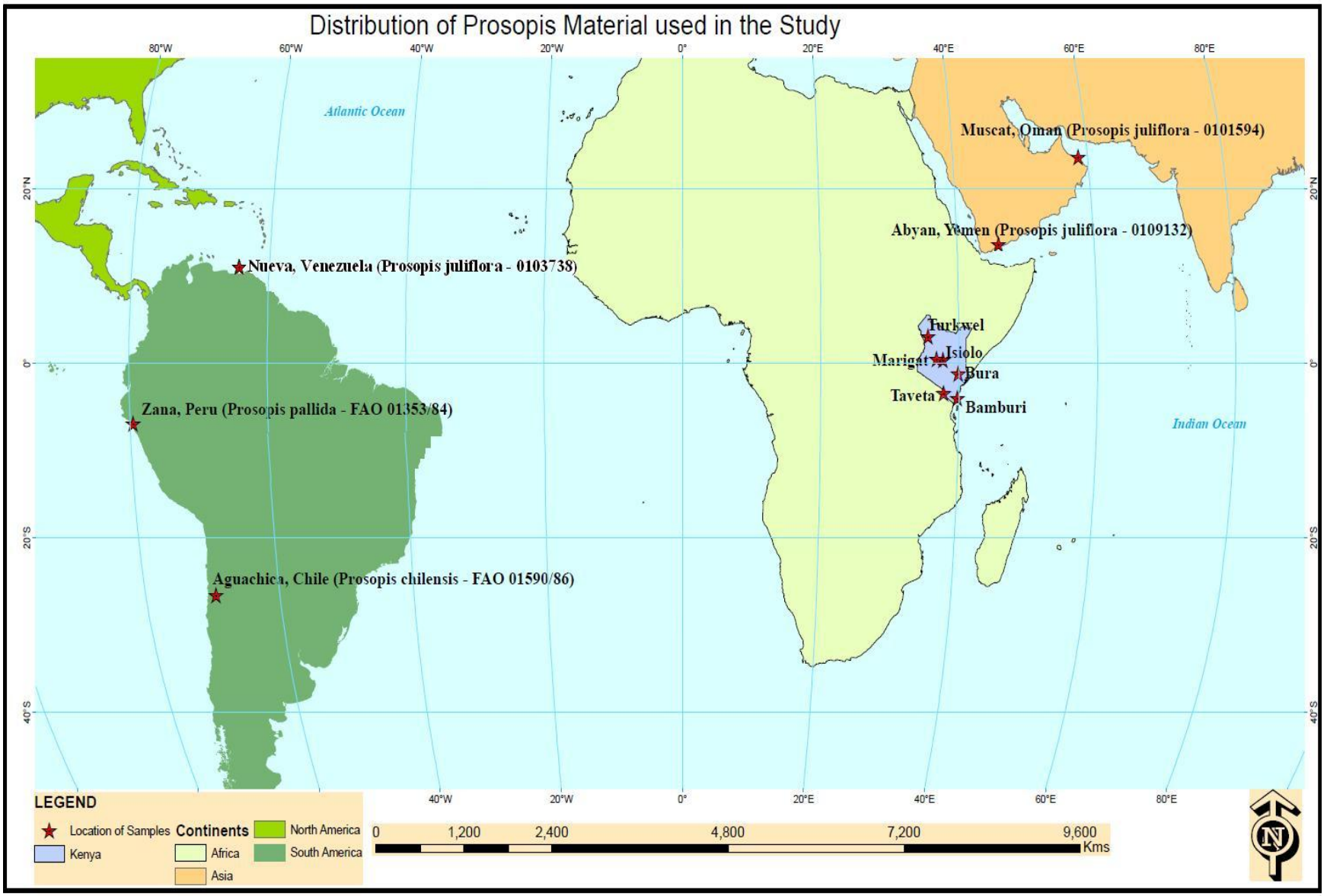


Figure 1. Geographical location of *Prosopis* species references and Kenyan *Prosopis* populations. Geographical distribution of the six Kenyan *Prosopis* populations and five *Prosopis* species references used in this study.

Table 2. Random amplified polymorphic DNA (RAPD) primers (primer code) used in the polymerase chain reaction (PCR), oligonucleotide primers base sequence (primer sequence), percentage content of guanine and cytosine bases in the primer [GC content (%)] and melting temperature (Tm °C) for each primer.

Primer code	Primer sequence	GC content (%)	Tm (°C)
KFP-1	GGC TCG TAC C	70	34
KFP-2	CGT CCG TCA G	70	34
KFP-3	GTT AGC GGC G	70	34
KFP-4	CGG AGA GTA C	60	32
KFP-5	CCT GGC GAG C	80	36
KFP-6	TCC CGA CCT C	70	34
KFP-7	CCA GGC GCA A	70	34
KFP-8	AGC CGC TGG T	70	34
KFP-9	GAC TGG AGC T	60	32
KFP-10	ACG GTG CGC C	80	36

Table 3. Sample size (N), number of loci per sample (L), percentage polymorphism in the sampled population (%P), number of population specific loci (PSL) and Nei's mean diversity estimates (H_e) of 11 *Prosopis* species populations based on the ten RAPD markers.

Provenance/species	N	L	% P	PSL	H_e
Isiolo	30	135	59.8	1	0.191
Marigat	30	114	47.0	0	0.140
Bamburi	30	113	46.6	2	0.127
Turkwel	18	98	41.6	1	0.148
Taveta	30	106	42.0	1	0.132
Bura	30	92	31.0	0	0.091
P. chilensis	30	112	46.6	1	0.144
P. pallida	30	92	22.1	0	0.069
P. juliflora 0101594	25	73	28.3	0	0.110
P. juliflora 0109132	27	66	21.0	0	0.077
P. juliflora 0103738	20	65	15.5	0	0.065

was carried out three times; (1) within and among the Kenyan *Prosopis* populations alongside the reference species, (2) within and among the Kenyan *Prosopis* populations, and (3) within and among the *P. juliflora* reference populations. AMOVA was used to estimate population differentiation directly from the RAPD molecular data. AMOVA and PCA were performed using GenAlEx 6.4 software (Peakall and Smouse, 2006).

RESULTS

All the six Kenyan populations and the reference species showed variation in polymorphism and mean expected heterozygosity (H_e) over the 10 primers (Table 3). Polymorphism was higher amongst the Kenyan populations (ranging from 31.0% in Bura to 59.8% in Isiolo) than that of the reference species (ranging from 15.5% in *P. juliflora* (0103738) to 46.6% in *P. chilensis*). Similarly, H_e range was higher in the Kenyan populations (ranging from 0.091 in Bura to 0.191 in Isiolo) than in the reference species (ranging from 0.065 in *P. juliflora* (0103738) to 0.144 in *P. chilensis*). The results for polymorphism and H_e (Table 3) were consistent with those of a PCA in which more genetically diverse populations had higher multi-dimensional spread, than populations with low genetic diversity (Figure 2). The PCA results separated the three references as *P. chilensis, P. juliflora* and *P. pallida* (Figure 2). For Kenyan populations, Bamburi and Taveta were differentiated as separate populations but the other four populations were closely interlinked (Figure 2). The PCA multidimensional spread revealed that Bamburi was closer to *P. pallida*, Taveta closer to *P. chilensis* and the other populations closer to both *P. chilensis and P. juliflora*. Cumulatively, the first three principal axes accounted for 64.9% of the genetic diversity found in the entire study material, for which the first axis contributed 28.5%, second axis contributed 20.5% and third axis contributed 15.9%.

Analysis of molecular variance (Table 4) revealed a higher variation (62%, $p < 0.001$) between the Kenyan *Prosopis* populations than within (38%, $p < 0.001$). A

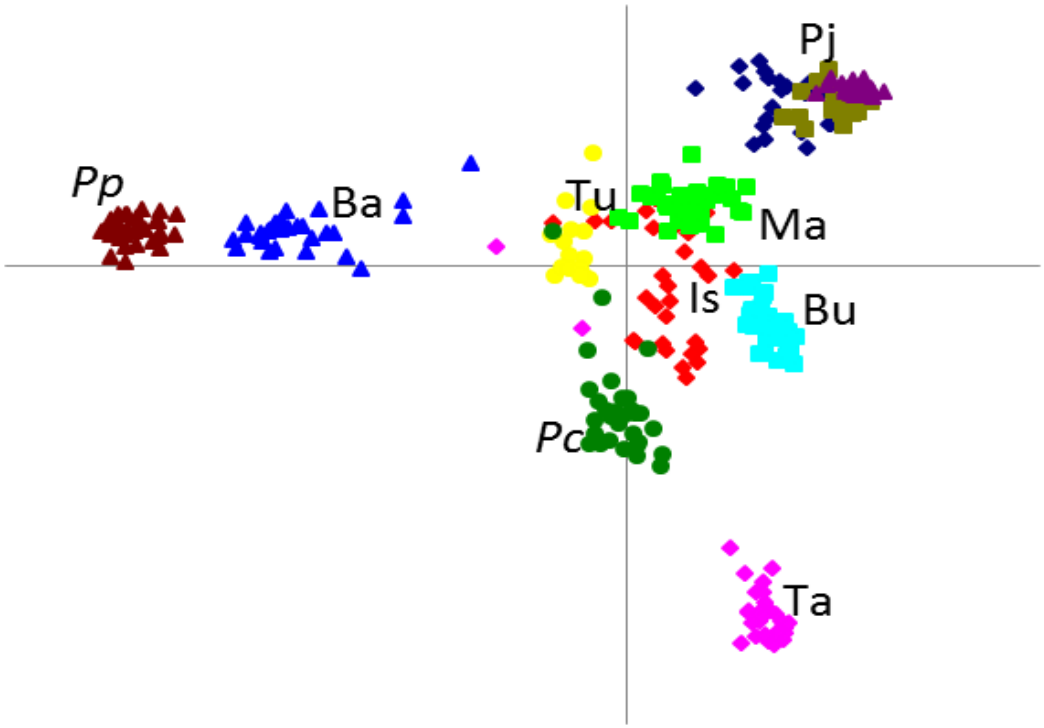


Figure 2. Distribution of tree samples of reference *Prosopis* species and Kenyan *Prosopis* populations along the first two principal component axes. Distribution of six Kenyan *Prosopis* populations; Bamburi (Ba, blue triangles), Bura (Bu, turquoise squares), Isiolo (Is, red diamonds), Marigat (Ma, green squares), Taveta (Ta, pink diamonds) and Turkwel (Tu, yellow circles); and reference specie; *P. chilensis* (Pc green circles), *P. juliflora* (Pj, blue diamonds, purple triangles and grey squares) and *P. pallida* (Pp, brown triangles) along the first two principal component axes. *P. juliflora* had three reference provenances. PCA was based on Orloci (1978) algorithm of distance matrix. The first axis accounted for 28.5% of genetic diversity and the second axis accounted 20.5% genetic diversity.

Table 4. Results of analysis of molecular variance (AMOVA) of six Kenyan *Prosopis* populations and three reference species (a), three *Prosopis juliflora* provenances (b), and six Kenyan *Prosopis* populations (c). Probability (P) values are based on 1000 random permutations of individuals across populations. DF = degrees of freedom.

Source of variation	df	Sum of squares	Mean sum of squares	Variance components	Variance (%)	*P*
(a) AMOVA results for six Kenyan *Prosopis* Population and three *Prosopis* reference species						
Among regions*	2	1753.747	876.874	2.725	7	<0.001
Among populations**	8	5006.386	625.798	22.641	61	<0.001
Within all population	289	3429.080	11.865	11.865	32	<0.001
Total	299	10189.213		37.232	100	
(b) AMOVA results for *Prosopis juliflora*						
Among provenances	2	981.456	490.728	20.297	74	<0.001
Within provenances	69	500.891	7.259	7.259	26	<0.001
Total	71	1482.347		27.557	100	
(c) AMOVA results for six Kenyan *Prosopis* populations						
Among populations	5	3191.747	638.349	22.418	62	<0.001
Within populations	162	2241.289	13.835	13.835	38	<0.001
Total	167	5433.036		36.254	100	

* Three distinct regions, Kenya (1), South America (2) and Middle East (3) where *Prosopis* material was sourced from; ** the pooling of all three *P. juliflora* provenances into a single population and treatment of all the other *Prosopis* samples as discrete populations.

Table 5. Private marker (specific locus) only amplified in four Kenyan population and *P. chilensis* (Population / Reference) using Random Amplified Polymorphic DNA (RAPD) markers (RAPD marker sequence). Marker code is the lab description of the primers used in the study.

Population/ reference	RAPD marker sequence	Marker code	Specific locus (bp)
Turkwel	GGCTCGTACC	KFP-1	200
P. chilensis	GGCTCGTACC	KFP-1	1300
Taveta	GTTAGCGGCG	KFP-3	1400
Bamburi	CGGAGAGTAC	KFP-4	750
Bamburi	CGGAGAGTAC	KFP-4	450
Isiolo	CCTGGCGAGC	KFP-5	230

similar trend was also observed in *P. juliflora* reference material where molecular variation was higher between (74%, $p < 0.001$) than within (26%, $p < 0.001$) populations. Three primers (KFP1, KFP3, KFP 4 and KFP5) generated molecular markers that were only found in four Kenyan populations and *P. chilensis* (Table 5). The specific molecular markers for Bamburi at 450 and 750 bp and contrasting absence of similar markers in Isiolo population are shown in Figure 3. Other primers showing specific markers were: KFP-1 for Turkwel population at 200 bp, and for *P. chilensis* at 1300 bp, KFP-3 for Taveta population at 1400 bp, and KFP-5 for Isiolo population at 230 bp (Table 5).

According to Nei's unbiased genetic distance matrix (Table 6), the most genetically close Kenyan populations were Isiolo and Marigat (0.172) whereas the most genetically distant materials were *P. pallida* and *P. juliflora* - 0103738 (0.463). Results also indicate that the three *P. juliflora* populations were genetically closer to each other than to the other two reference species (Table 6). A dendogram based on Nei's unbiased genetic distance (Figure 4) revealed clustering of *P. chilensis* with Taveta, *P. juliflora* with Bura, Isiolo and Marigat, *P. pallida* with Bamburi, whereas the Turkwel population was between *P. chilensis* and *P. juliflora* (Figure 4). Five out of six Kenyan populations clustered with reference species (Figure 4), thus facilitating comparison of genetic diversity of local populations with the corresponding reference species from the natural range. The Kenyan *P. juliflora* populations in Isiolo and Marigat had a higher genetic diversity than the reference *P. juliflora* with which they clustered. Similarly, the Kenyan Bamburi population had a higher genetic diversity than *P. pallida* with which it clustered. In contrast, genetic diversity of Bura population was lower than the reference *P. juliflora* with which it clustered, and that of Taveta population lower than *P. chilensis* with which it clustered.

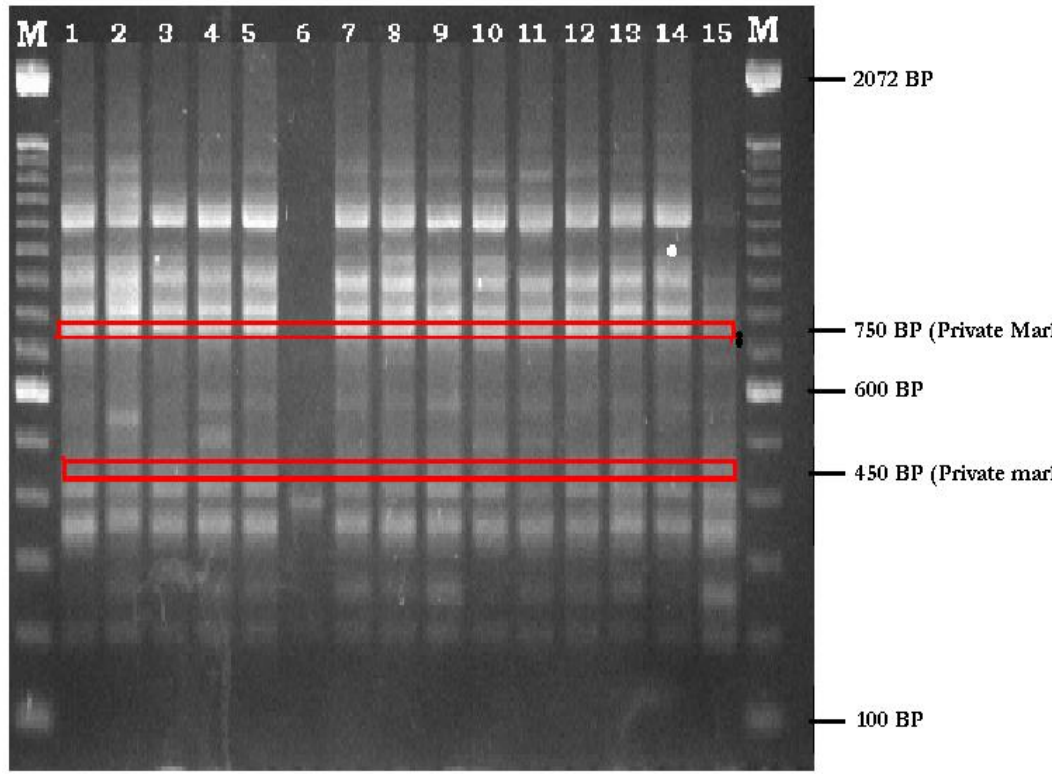
DISCUSSION

The aim of this study was to determine genetic diversity and species composition of six *Prosopis* populations in Kenya. Results show that genetic variation was larger amongst populations than within populations. The relative genetic isolation of these populations is also supported by the presence of unique genetic markers for some populations generated by the four primers (Table 5).

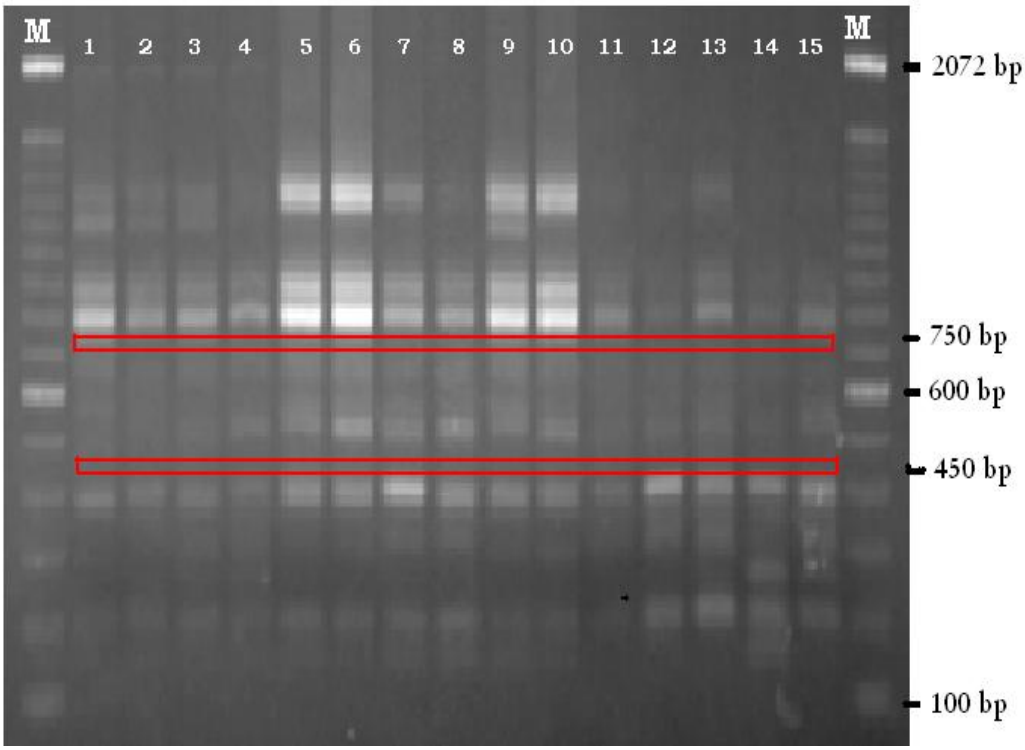
Genetic diversity of *Prosopis* populations in Kenya

Our first hypothesis was that the naturally established *Prosopis* populations in Kenya had a high genetic diversity because of multiple introductions of species and provenances within sites. Five out of six Kenyan populations clustered with reference species (Figure 4), thus facilitating comparison of genetic diversity between local populations with their corresponding references from the natural range. For three out of these five populations, genetic diversity was indeed higher than populations of the species from the native range. Moreover, genetic diversity for all *P. juliflora* populations from Kenya was higher than populations introduced to the Middle East (Oman, Yemen). These findings support our hypothesis that *Prosopis* populations in Kenya have a high genetic diversity, as a result of multiple introductions. Our findings are also consistent with increase of genetic diversity with multiple species introductions (Pairon et al., 2010), as formerly separated genotypes mixed and hybridized (Schierenbeck and Ellstrand, 2009, Parsons et al., 2011). The high genetic diversity found in *P. juliflora* populations can be explained by the introduction of several *P. juliflora* provenances to Kenya and subsequent seed exchange in the country (Maghembe et al., 1983; Otsamo et al., 1993).

Partitioning of genetic variation in the studied *Prosopis* germplasm was based on two population genetics assumptions (Wright, 1976), as origin of *Prosopis* germplasm introduced to Kenya and Middle East was unknown; some materials may have originated from the reference provenances from the natural range. First, each of the study material had population characteristics; such a free random mating of individuals within a population with minimal or no inter-population gene flow. Second, there existed three distinct (geographically



(a)



(b)

Figure 3. Private markers generated by primer KFP 4 at 450 and 750 bp for Bamburi population (a) and absence of such markers for the same primer for Isiolo population, (b) A 1.4% agarose gel stained in ethidium bromide showing two private bands (markers) at loci 750 and 450 bp in Bamburi population, (a) and Isiolo population where the private marker were missing in the same loci (b). M is 100 bp molecular weight marker (Invitrogen, UK) whereas 1 to 15 are the tree samples.

Table 6. Pairwise population matrix of Nei's unbiased genetic distance (Nei, 1978) of six Kenyan *Prosopis* populations and five *Prosopis* references (population/ reference). The Kenyan populations are described by their location names, whereas references are denoted as Pp (*P. pallida*), Pc (*P. chilensis*), Pj1 (*Prosopis juliflora*- 0101594), Pj2 (*P. juliflora*-0109132) and Pj3 (*P. juliflora* - 0103738).

Population/ reference	Isiolo	Marigat	Bamburi	Turkwel	Taveta	Bura	Pp	Pc	Pj1	Pj2	Pj3
Isiolo	0.000										
Marigat	0.172	0.000									
Bamburi	0.248	0.233	0.000								
Turkwel	0.266	0.282	0.315	0.000							
Taveta	0.262	0.287	0.367	0.354	0.000						
Bura	0.223	0.149	0.310	0.323	0.290	0.000					
Pp	0.337	0.314	0.217	0.385	0.425	0.398	0.000				
Pc	0.217	0.197	0.251	0.318	0.234	0.221	0.328	0.000			
Pj1	0.229	0.181	0.309	0.335	0.312	0.273	0.379	0.267	0.000		
Pj2	0.230	0.210	0.328	0.309	0.317	0.260	0.429	0.287	0.187	0.000	
Pj3	0.300	0.253	0.421	0.378	0.367	0.322	0.463	0.341	0.222	0.262	0.000

isolated) regions for *P. juliflora* (Kenya, South America and Middle East). The assumption was based on the expectation that further genetic differentiation or evolution occurs after the materials were introduced to Kenya and Middle East, thus leading to genetic variation from their progenitors. Comparisons of genetic variation partitioning across the three regions (Kenya, Middle East and South America) revealed a higher genetic variation among *Prosopis* populations (61%) than within *Prosopis* populations (32%). The trend was also found for Kenyan population where genetic variation among populations was higher (62%) than genetic variation within populations (38%). Our results were consistent with genetic variation partitioning among *Prosopis* species in their natural range (Juarez-Munoz et al., 2002). The genetic variation of 7% attributed to geographical regions in this study was higher than 3% attributed to the geographical regions within the natural range of *Prosopis* species (Ferreyra et al., 2010). This may be relegated to a further environmentally driven genetic differentiation of introduced germplasm (Ferreyra et al., 2010), hybridization (Vega and Hernandez, 2005; Landeras et al., 2006) and polyploidy (Trenchard et al., 2008) that may infer higher genetic variability between introduced genotypes and their progenitors.

The higher genetic variation found among *P. juliflora* populations (74%) than genetic variation within *P. juliflora* populations (26%) contrasts with genetic variation of *P. juliflora* populations introduced to Sudan (Hamza, 2010), where genetic variation among populations (33%) was lower than genetic variation within populations (67%). The most likely reason for the contrast between our study and that of Sudan is that populations sampled in Sudan were closer to each other than those sampled in Kenya. Geographical proximity may facilitate inter-population gene flow, thus reducing the genetic diversity among populations.

Both the larger genetic variation amongst populations than within populations and the occurrence of private markers in some study populations indicate genetic differentiation amongst the Kenyan populations. Such genetic differentiation can be either as a result of genetic variation of germplasm at introduction, or a gradual adaptation of populations to site-specific environmental conditions.

Do *Prosopis* populations consist of several species?

We also hypothesized that naturally established stands consist of a mixture of species and/or hybrids, as several species were introduced within sites, and subsequent seed dispersal was random among sites. The distribution of Kenyan populations and reference species along the first two PCA axes and their clustering in UPGMA dendogram did not reveal any evidence of establishment of a mixture of species or species and hybrids within any one site. Therefore, our hypothesis was rejected. Instead, the results suggest that only *P. juliflora* was present at Bura and Marigat, despite the fact that also *P. chilensis* and *P. pallida* were introduced in Bura and Marigat (Otsamo et al., 1993; Rosenschein et al., 1999). Similarly, only *P. pallida* was successfully established at Bamburi, although also *P. juliflora* was also introduced at this site (Maghembe et al., 1983). In Turkwel, neither *P. chilensis* nor *P. juliflora* were successfully established, despite the introduction of the two species in this site (Oba et al., 2001).

Our study confirms past description of populations at Bura, Isiolo and Marigat as *P. juliflora*, (Ngunjiri and Choge, 2004; Mwangi and Swallow, 2008) and Bamburi as *P. pallida* (Trenchard et al., 2008). However, the results suggest that the Taveta population is not *P. juliflora,* as proposed by Ngunjiri and Choge (2004), but is likely to be *P. chilensis.* The Turkwel population seems to be neither *P. chilensis,* as proposed by Stave et al. (2003)

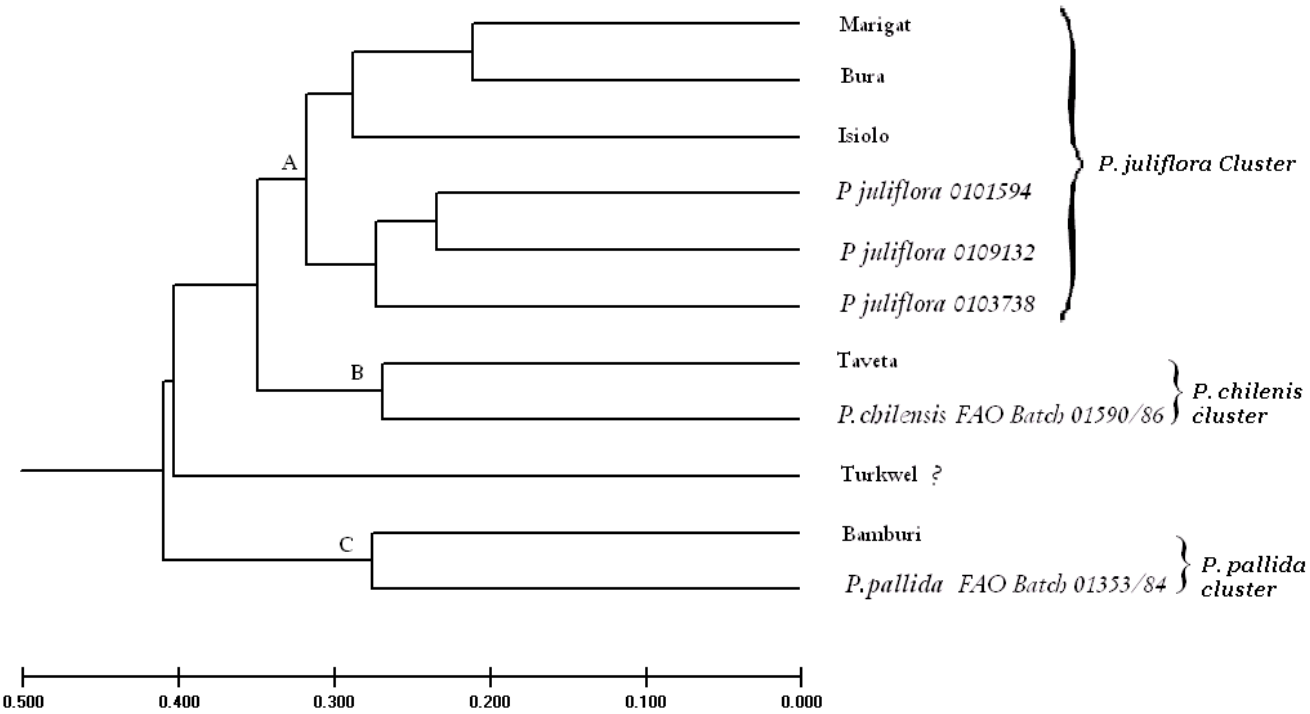


Figure 4. Clustering of reference *Prosopis* species with six Kenyan *Prosopis* populations. Dendrogram of unweighted pair-wise group arithmetic averaging (UPGMA) cluster analysis of three *Prosopis* reference species (*P. chilensis, P. juliflora* and *P. pallida*) and six Kenyan *Prosopis* species populations based on Nei's (1978) unbiased genetic distance. *Prosopis juliflora* is supported by node A, *P. chilensis* by node B and *P. pallida* by node C. The Kenyan populations clustered with *P. juliflora* (Marigat, Bura and Isiolo), *P. chilensis* (Taveta) and *P. pallida* (Bamburi). Turkwel population appeared to be a *P. chilenis* – *P. juliflora* hybrid.

nor *P. juliflora* as proposed by Trenchard et al. (2008), but instead we demonstrated that it could be a *P. chilensis* - *P juliflora* hybrid, as both species were indeed introduced into the area (Oba et al., 2001) and hybridization between *P. chilensis* and other species in Algarobia section is quite common (Hunziker et al., 1986; Landeras et al., 2006; Sherry et al., 2011). Besides the clustering of Kenyan populations with reference species, our findings also corroborate differentiation of *P. juliflora* and *P. pallida* by RAPD markers (Landeras et al., 2006; Sherry et al., 2011). Our study has contributed to increasing evidence for molecular differentiation of *P. juliflora* and *P. pallida* which are morphologically described as a complex (Pasiecznik et al., 2001).

Several species were introduced to the Bamburi, Bura, Marigat and Turkwel sites but surprisingly, our study reveal the occurrence of a single species or a single hybrid at each site. Four populations (Bamburi, Isiolo, Taveta and Turkwel) had a specific private marker each, suggesting their unique genetic differentiation. Three inferences can be made from these results. First, not all introduced species were adapted, as species mixtures were not found at any one site where mixture of species were introduced. Second, natural random seed dispersal (Mwangi and Swallow, 2008; Mworia et al., 2011) or exchange of germplasm between sites (Kaarakka et al., 1990; Otsamo et al., 1993) did not seem to induce genetic homogenization, as implied by the genetic uniqueness in four of the studied populations. Third, adaptation of the successfully established germplasm from the introduced pool was site specific, probably because of variation of environmental factors among sites. *P. juliflora* was a common specie in two sites (Bura and Marigat) and a parent of the hybrid in Turkwel, yet the three sites are within the most *Prosopis* invaded areas of Kenya (Stave et al., 2003; Ngunjiri and Choge, 2004; Mwangi and Swallow, 2008). Therefore, we opine that *P. juliflora* and its hybrids are among the most aggressive invaders.

Conclusion

The multiple introductions of species and provenances within sites in Kenya have led to high genetic *Prosopis* diversity and hybridization. Although, several species were introduced to some sites, only a single species or a hybrid was successfully adapted in any given site whereas, it is generally assumed that naturally established *Prosopis* populations in Kenya consist almost entirely of *P. juliflora*. We revealed the presence of *P. chilensis, P. pallida* and a likely hybrid between *P. chilensis* and *P. juliflora*. Our study classified the Kenyan *Prosopis* populations as *P. chilensis* for Taveta,

P. juliflora for Bura, Isiolo and Marigat, *P. pallida* for Bura and a likely *P. chilensis – P. juliflora* hybrid for Turkwel. We have further revealed genetic differentiation of Kenyan *Prosopis* populations as evident from specific molecular markers.

ACKNOWLEDGEMENTS

This study was financially supported by a NUFFIC PhD grant No CF3671/2007 and a KEFRI research grant to Gabriel Muturi. Seeds of *P. chilensis* and *P. pallida* were provided by Danish Centre for Forest, Landscape and Planning - courtesy of Dr. Lars Schmidt. Seeds of the three *P. juliflora* reference provenances were obtained from Royal Botanical Gardens, of UK, Courtesy of Dr. Timothy Pearce and Dr. Janet Terry. The staff of KEFRI's Biotechnology laboratory provided valuable support in the field, greenhouse and laboratory during plant sampling, production of reference material seedlings and molecular analysis. We appreciate the contribution of all institutions and individuals who supported this study.

REFERENCES

Anderson S (2005). Spread of the introduced tree species *Prosopis juliflora* (Sw.) DC, in the lake Baringo area, Kenya. Institutionen for Skoglig vegetationsekologi. ISSN 1652-4918. p. 31

Bessega C, Ferreyra L, Julio N, Montoya S, Saidman B, Vilardi JC (2000). Mating system parameters in species of genus *Prosopis* (Leguminosae). Hereditas 132:19-27.

Burkart A (1976). A monograph of the genus *Prosopis* (Leguminosae subfam. Mimosoideae). J. Arnold. Arbr. Harv. Univ. 5:219-249, 450-525.

Edwards K, Johnstone C, Thompson C (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19:1349.

Ferreyra LI, Vilardi JC, Tosto DS, Julio NB, Saidman BO (2010). Adaptive genetic diversity and population structure of the "algarrobo" [*Prosopis chilensis* (Molina) Stuntz] analysed by RAPD and isozyme markers. Eur. J. For. Res. 129:1011-1025.

Geiger JH, Pratt PD, Wheeler GS, Williams DA (2011). Hybrid vigor for the invasive exotic Brazilian pepper tree (*Schinus terebinthifolius* Raddi., Anacardiaceae) in Florida. Int. J. Plant Sci. 172:655-663.

Government of the Republic of Kenya (GoK) (2007). Kenya Vision 2030: A globally competitive and prosperous Kenya, p. 164.

Hamza BN (2010). Genetic variation within and among three invasive *Prosopis juliflora* (Leguminosae) populations in the River Nile State, Sudan. Int. J. Gen. Mol. Biol. 2:92-100.

Harris PJC, Pasiecznik NM, Smith SJ, Billington JM, Ramirez L (2003). Differentiation of *Prosopis juliflora* (Sw.) DC. and *P. pallida* (H. & B. ex. Willd.) HBK using foliar characters and ploidy. For. Ecol. Manag. 180:153-164.

Hauser LA, Crovello TJ (1982). Numerical-Analysis of Generic Relationships in Thelypodieae (Brassicaceae). Syst. Bot. 7:249-268.

Hunziker JH, Saidman BO, Naranjo CA, Palacios RA, Poggio L, Burghardt AD (1986). Hybridization and Genetic-Variation of Argentine Species of *Prosopis*. For. Ecol. Manag. 16:301-315.

Juarez-Munoz J, Carrillo-Castaneda G, Arreguin R, Rubluo A (2002). Inter- and intra-genetic variation of four wild populations of *Prosopis* using RAPD-PCR fingerprints. Biodivers. Conserv. 11:921-930.

Kaarakka V, Johansson S, Luukkanen O, Maingi J (1990). Forestry in Irrigation schemes II. Research activities at Bura, Kenya 1988-1989. Tropical Forestry Reports No 5. University of Helsinki.

Landeras G, Alfonso M, Pasiecznik NM, Harris PJC, Ramirez L (2006). Identification of *Prosopis juliflora* and *Prosopis pallida* provenances using molecular markers. Biodivers. Conserv. 15:1829-1844.

Lockwood JL, Cassey P, Blackburn TM (2009). The more you introduce the more you get: the role of colonization pressure and propagule pressure in invasion ecology. Divers. Distrib. 15:904-910.

Machua J, Muturi G, Omondi SF, Gicheru J (2011). Genetic diversity of Jatropha curcas L. populations in Kenya using RAPD molecular markers: Implication to plantation establishment. Afr. J. Biotechnol. 10:3062-3069.

Maghembe JA, Kariuki EM, Haller RD (1983). Biomass and nutrient accumulation in young *Prosopis juliflora* at Mombasa, Kenya. Agrofor. Syst. 1:313-321.

Miller MP (1997). Tools for population genetic anlyses (TFPGA) 1.3 A Windows program for the analysis of allozyme and molecular population genetic data.

Mwangi E, Swallow B (2008). *Prosopis juliflora* invasion and rural livelihoods in the Lake Baringo area of Kenya. Conserv. Soc. 6:130-140.

Mworia JK, Kinyamario JI, Omari JK, Wambua JK (2011). Patterns of seed dispersal and establishment of the invader *Prosopis juliflora* in the upper floodplain of Tana River, Kenya. Afr. J. Range Fora. Sci. 28:35-41.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a smaller number of individuals. Genetics 89:583-590.

Ngunjiri FD, Choge SK (2004). Status and impact of *Prosopis* species in Kenya. In Choge S.K. and Chikamai, B.N. (eds) Proceedings of the workshop on integrated management of *Prosopis* species in Kenya. KEFRI.

Oba G, Nordal I, Stenseth NC, Stave J, Bjora CS, Muthondeki JK, Bii WKA (2001). Growth performance of exotic and indigenous tree species in saline soils in Turkana, Kenya. J. Arid Environ. 47:499-511.

Olukoye GA, Wamicha WN, Kinyamario JI (2003). Assessment of the performance of exotic and indigenous tree and shrub species for rehabilitating saline soils of Northern Kenya. Afr. J. Ecol. 41:164-170.

Orloci L (1978). Multivariate analysis in vegetation research. The Hague: Dr W. Junk B. V. p. 451

Otsamo A, Laxen J, Johansson S, Kaarakka V, Kuusipalo, O, Luukkanen O, Maua JO (1993). Forest research in Bura, Kenya 1984-1993. Final report of Research component in the Bura fuelwood project. University of Helsinki, Helsinki, p. 136.

Pairon M, Petitpierre B, Campbell M, Guisan A, Broennimann O, Baret PV, Jacquemart AL, Besnard G (2010). Multiple introductions boosted genetic diversity in the invasive range of black cherry (*Prunus serotina*; Rosaceae). Ann. Bot. 105:881-890.

Parsons KJ, Son YH, Albertson RC (2011). Hybridization Promotes Evolvability in African Cichlids: Connections Between Transgressive Segregation and Phenotypic Integration. Evol. Biol. 38:306-315.

Pasiecznik NM, Felker P, Harris PJC, Harsh LN, Cruz G, Tewarri JC, Cadoret K, Maldonado, LJ (2001). The *Prosopis juliflora-Prosopis pallida* complex: A monograph. HDRA.

Peakall R, Smouse PE (2006). GenALEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Nat. 6:288-295.

Pizzonia J (2001) Electrophoresis gel image processing and analysis using the KODAK 1D software. Biotechniques 30(6):1316-1320.

Rieseberg LH, Kim SC, Randell RA, Whitney KD, Gross BL, Lexer C, Clay K (2007). Hybridization and the colonization of novel habitats by annual sunflowers. Genetica 129:149-165.

Rosenschein A, Tietema T, Hall DO (1999). Biomass measurement and monitoring of trees and shrubs in a semi-arid region of central Kenya. J. Arid Environ. 42:97-116.

Ross CA, Auge H (2008). Invasive Mahonia plants outgrow their native relatives. Plant Ecol. 199:21-31.

Saidman BO, Vilardi JC, Pocovi MI, Acreche N (1996). Genetic divergence among species of the section Strombocarpa, genus *Prosopis* (Leguminosae). J. Gen. 75:139-149.

Schierenbeck KA, Ellstrand NC (2009). Hybridization and the evolution of invasiveness in plants and other organisms. Biol. Invas. 11:1093-1105.

Sherry M, Smith S, Patel A, Harris P, Hand P, Trenchard L, Henderson J (2011). RAPD and microsatellite transferability studies in selected

species of *Prosopis* (section Algarobia) with emphasis on *Prosopis juliflora* and *P. pallida*. J. Gen. 90:251-264.
Simberloff D (2009). The role of propagule pressure in biological invasions. In: Annual Rev. Ecol. Evol. Syst. 40:81-102.
Sneath PHA, Sokal RR (1973). Numerical taxonomy. Freeman San Fransisco, USA. p. 573.
Stave J, Oba G, Bjora CS, Mengistu Z, Nordal I, Stenseth NC (2003). Spatial and temporal woodland patterns along the lower Turkwel River, Kenya. Afr. J. Ecol. 41:224-236.
Trenchard LJ, Harris PJC, Smith SJ, Pasiecznik NM (2008). A review of ploidy in the genus *Prosopis* (Leguminosae). Botan. J. Linn. Soc. 156:425-438.
van Klinken RD (1999). Developmental host-specificity of *Mozena obtusa* (Heteroptera : Coreidae), a potential biocontrol agent for *Prosopis* species (Mesquite) in Australia. Biol. Control 16:283-290.
Vega MV, Hernandez P (2005). Molecular evidence for natural interspecific hybridization in *Prosopis*. Agrofor. Syst. 64:197-202.
Wright JW (1976). Introduction to forest genetics. Academic Press Inc., USA p. 463.
Yeh FC, Yang R, Boyle T (1999). Popgene Version 1.32. Ag/For Molecular biology and biotechnology centre, University of Alberta and Center for International Forestry Research, Alberta, Canada, p. 28.
Zimmerman HG (1991). Biological control of mesquite, *Prosopis* spp. (Fabaceae), in South Africa. Agric. Ecosyst. Environ. 37:175-186.